THE USE OF COPOLYMER 1 AND RELATED PEPTIDES AND POLYPEPTIDES AND T CELLS TREATED THEREWITH FOR NEUROPROTECTIVE THERAPY

FIELD OF THE INVENTION

The present invention relates to compositions and methods for the promotion of nerve regeneration or prevention or inhibition of neuronal degeneration to ameliorate the effects of injury or disease of the nervous system (NS). In particular, the invention relates to compositions comprising Copolymer 1 (Cop 1) or a Cop 1-related peptide or polypeptide, and/or activated T cells treated with Cop 1 or a Cop 1-related peptide or polypeptide, to promote nerve regeneration or to prevent or inhibit neuronal degeneration caused by injury or disease of nerves within the central nervous system or peripheral nervous system of a human subject. The compositions of the present invention may be administered alone or may be optionally administered in any desired combination.

BACKGROUND OF THE INVENTION

The nervous system comprises the central and the peripheral nervous system. The central nervous system (CNS) is composed of the brain and spinal cord; the peripheral nervous system (PNS) consists of all of the other neural elements, namely the nerves and ganglia outside of the brain and spinal cord.

Damage to the nervous system may result from a traumatic injury, such as penetrating trauma or blunt trauma, or a disease or disorder, including but not limited to

Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS), diabetic neuropathy, senile dementia, and ischemia.

Maintenance of central nervous system integrity is a complex "balancing act" in which compromises are struck with the immune system. In most tissues, the immune system plays an essential part in protection, repair, and healing. In the central nervous system, because of its unique immune privilege, immunological reactions are relatively limited (Streilein, 1993, 1995). A growing body of evidence indicates that the failure of the mammalian central nervous system to achieve functional recovery after injury is a reflection of an ineffective dialog between the damaged tissue and the immune system. For example, the restricted communication between the central nervous system and blood-borne macrophages affects the capacity of axotomized axons to regrow; transplants of activated macrophages can promote central nervous system regrowth (Lazarov Spiegler et al, 1996; Rapalino et al, 1998).

Activated T cells have been shown to enter the central nervous system parenchyma, irrespective of their antigen specificity, but only T cells capable of reacting with a central nervous system antigen seem to persist there (Hickey et al, 1991; Werkele, 1993; Kramer et al, 1995). T cells reactive to antigens of central nervous system white matter, such as myelin basic protein (MBP), can induce the paralytic disease experimental autoimmune encephalomyelitis (EAE) within several days of their inoculation into naive recipient rats (Ben-Nun, 1981a). Anti-MPB T cells may also be involved in

the human disease multiple sclerosis (Ota, K. et al, 1990; Martin, 1997). However, despite their pathogenic potential, anti-MBP T cell clones are present in the immune systems of healthy subjects (Burns, 1983; Pette, M. et al, 1990; Martin et al, 1990; Schluesener et al, 1985). Activated T cells, which normally patrol the intact central nervous system, transiently accumulate at sites of central nervous system white matter lesions (Hirschberg et al, 1998).

A catastrophic consequence of central nervous system injury is that the primary damage is often compounded by the gradual secondary loss of adjacent neurons that apparently were undamaged, or only marginally damaged, by the initial injury (Faden et al, 1992; Faden 1993; McIntosh, 1993). The primary lesion causes changes in extracellular ion concentrations, elevation of amounts of free radicals, release of neurotransmitters, depletion of growth factors, and local inflammation. These changes trigger a cascade of destructive events in the adjacent neurons that initially escaped the primary injury (Lynch et al, 1994; Bazan et al, 1995; Wu et al, 1994). This secondary damage is mediated by activation of voltage-dependent or agonist-gated channels, ion leaks, activation of calcium-dependent enzymes such as proteases, lipases and nucleases, mitochondrial dysfunction and energy depletion, culminating in neuronal cell death (Yoshina et al, 1991; Hovda et al, 1991; Zivin et al, 1991; Yoles et al, 1992). The widespread loss of neurons beyond the loss caused directly by the primary injury has been called "secondary degeneration."

Another tragic consequence of central nervous system injury is that neurons in the mammalian central nervous system do not undergo spontaneous regeneration following an injury. Thus, a central nervous system injury causes permanent impairment of motor and sensory functions.

Spinal cord lesions, regardless of the severity of the injury, initially result in a complete functional paralysis known as spinal shock. Some spontaneous recovery from spinal shock may be observed, starting a few days after the injury and tapering off within three to four weeks. The less severe the insult, the better the functional outcome. The extent of recovery is a function of the amount of undamaged tissue minus the loss due to secondary degeneration. Recovery from injury would be improved by neuroprotective treatment that could reduce secondary degeneration.

In the laboratory of the present inventors, it has recently been discovered that activated T cells that recognize an antigen of the nervous system (NS) of the patient promote nerve regeneration or confer neuroprotection. Reference is made to PCT publication WO 99/60021, the entire contents of which is hereby incorporated herein by reference. Until recently, it had been thought that the immune system excluded immune cells from participating in nervous system repair. It was quite surprising to discover that NS-specific activated T cells could be used to promote nerve regeneration or to protect nervous system tissue from secondary degeneration which may follow damage caused by injury or disease of the CNS or PNS.

NS-specific activated T cells as described in said WO 99/60021 publication are activated T cells having specificity for an antigen of the NS of a patient. The antigen used to confer the specificity to the T cells may be a self NS-antigen of the patient, a peptide derived therefrom, or an NS-antigen of another individual or even another species, or a peptide derived therefrom, as long as the activated T cell recognizes an antigen in the NS of the patient.

Said NS-specific activated T cells are for use to promote nerve regeneration or to prevent or inhibit the effects of disease. If the disease being treated is an autoimmune disease, in which the autoimmune antigen is an NS antigen, the T cells which are used for the treatment of neural damage or degeneration caused by such disease are not activated against the same autoimmune antigen involved in the disease.

The above-rererenced PCT publication WO 99/60021 discloses that therapy for amelioration of effects of injury or disease comprising administration of NS-specific activated T cells may optionally be in combination with an NS-specific antigen or peptide derived therefrom. An NS-specific antigen as defined in said WO 99/60021 refers to an antigen that specifically activates T cells such that following activation, the activated T cells accumulate at a site of injury or disease in the NS of the patient. Furthermore, oral administration of NS-specific antigen or a peptide derived

therefrom can be combined with active immunization to build up a critical T cell response immediately after injury.

In this prior invention, the NS-specific antigen used to activate the T cells *in vitro* or *in vivo* or to immunize the patient, may be an antigen obtained from NS tissue, preferably from tissue at a site of CNS injury or disease. Natural or synthetic NS-specific antigens or epitopes were disclosed to include MBP, MOG, PLP, MAG, S-100, β-amyloid, Thy-1, P0, P2 and a neurotransmitter receptor. Specific illustrative examples of such useful NS-specific antigens disclosed in WO 99/60021 are human MPB, human proteolipid protein, and human oligodendrocyte glycoprotein. Also disclosed were peptides derived from NS-specific, self-antigens or derivatives of NS-specific antigens that activate T cells, but do not induce an autoimmune disease, such as a peptide comprising amino acids 51-70 of myelin basic protein (MBP).

A high molecular weight synthetic basic random copolymer consisting of L-Ala, L-Glu, L-Lys and L-Tyr residues in the molar ratio of about 6 parts Ala to 2 parts Glu to 4.5 parts Lys to 1 part Tyr, and having a molecular weight of 15,000-25,000, was first described in US Patent No. 3,849,550 as an agent for treatment or prevention of experimental allergic encephalomyelitis (EAE), a disease resembling multiple sclerosis (MS) that can be induced in susceptible animals. Batches of this copolymer of average molecular weight 23,000, designated Copolymer 1 or Cop 1, were shown to

be highly effective in protecting and suppressing EAE in several animal species (Teitelbaum et al, 1971, 1974a, 1974b).

Later, Cop 1 was found to significantly reduce the number of relapses in patients with the exacerbating-remitting form of MS (Bornstein et al, 1990; Sela et al, 1990; Johnson et al, 1994). Copolymer 1, in the form of the acetate salts of synthetic polypeptides containing L-Glu, L-Ala, L-Tyr and L-Lys with an average molar fraction of 0.141, 0.427, 0.095 and 0.338, is the active ingredient of COPAXONE®, a medicament for the treatment of multiple sclerosis.

The mechanism underlying the therapeutic activity of Cop 1 in MS has been extensively studied. Cop 1 was found to be immunologically cross-reactive with myelin basic protein (MBP), the main autoantigen in EAE and MS. Its suppressive effect results from several mechanisms, such as inhibition of the autoreactive pathogenic T-cells on one hand (Teitelbaum et al, 1988) and stimulation of suppressor cells on the other hand (Aharoni et al, 1993). The first step essential for the activation of these specific processes is the binding of Cop 1 to the histocompatibility molecules. Indeed, it has been shown that Cop 1, in two different batches of molecular weight 5,550 and 8,600, and relative molar ratio of L-Ala (4.1-5.8 residues), L-Glu (1.4-1.8 residues), L-Lys (3.2-4.2 residues) and L-Tyr (1 residue), binds very efficiently to a variety of MHC class II molecules of mouse and human origin, and furthermore competes with MBP and its major epitope p84-102 for MHC binding and can even displace such antigens that had already been bound to the MHC molecule (Fridkis-Hareli et al,

1994). Direct evidence for interaction of Cop 1 with purified DR molecules has been reported, along with the suggestion that its effectiveness in EAE and MS is directly related to its binding in the group of HLH-DR proteins (Fridkis-Hareli et al, 1998). Furthermore, direct evidence has also been reported both for competitive interaction of Cop 1 and related copolymers and Collagen II C(II) peptide with rheumatoid arthritis (RA)-associated HLA-DR molecules and for inhibition of CII-specific T cell responses, suggesting that these compounds may be effective against rheumatoid arthritis.

Oral administration of autoantigen in order to obtain "oral tolerance" has been disclosed for the treatment of various autoimmune diseases. For example, EP 359 783 discloses the oral administration of MBP for the treatment of multiple sclerosis. PCT International Publications WO 91/12816, WO 91/08760 and WO 92/06704 all disclose the treatment of other autoimmune diseases using the oral tolerance method with a variety of autoantigens. Treatment of multiple sclerosis by ingestion or inhalation of Copolymer 1 has been disclosed in PCT publication WO 98/30227.

Compounds related to Copolymer 1 have also been studied and found to have properties similar to Copolymer 1. For example, copolymers composed of three of the four amino acids found in Copolymer 1 bind to purified Class II MHC molecules (Fridkis-Hareli et al, 1999a). In addition, binding motifs of Copolymer 1 to multiple sclerosis- and rheumatoid arthritis-associated HLA-DR molecules have recently been elucidated (Fridkis-Hareli et al, 1999b). From these binding

motifs, polypeptides of fixed sequence can readily be proposed and tested for binding to the peptide binding groove of the HLA-DR molecules. Such peptides would be expected to act in a way similar to Cop 1 itself.

Citation or identification of any reference in this section or any other part of this application shall not be construed as an admission that such reference is available as prior art to the invention.

SUMMARY OF THE INVENTION

The present invention is directed to methods and compositions for the promotion of nerve regeneration or prevention or inhibition of neuronal degeneration to ameliorate the effects of injury to, or disease of, the nervous system (NS). The present invention is based in part on the applicants' unexpected discovery that activated T cells against Cop 1 promote nerve regeneration or confer neuroprotection. As used herein, "neuroprotection" refers to the prevention or inhibition of degenerative effects of injury or disease in the NS. Until recently, it was thought that the immune system excluded immune cells from participating in nervous system repair. It was quite surprising to discover that Cop 1 activated T cells can be used to promote nerve regeneration or to protect nervous system tissue from secondary degeneration which may follow damage caused by injury or disease of the CNS or PNS.

"Activated T cell" as used herein includes (i) T cells that have been activated by exposure to Cop 1 or a Cop

1-related peptide or polypeptide and (ii) progeny of such activated T cells.

In one embodiment, the present invention provides pharmaceutical compositions comprising a therapeutically effective amount of Cop 1-specific activated T cells and methods for using such compositions to promote nerve regeneration or to prevent or inhibit neuronal degeneration in the CNS or PNS, in an amount which is effective to ameliorate the effects of an injury or disease of the NS. "Cop 1-specific activated T cells" as used herein refers to activated T cells having specificity for Cop 1 or a Cop 1-related peptide or polypeptide.

The Cop 1-specific activated T cells are used to promote nerve regeneration or to prevent or inhibit the secondary degenerative effects which may follow primary NS injury or the effects of neurodegenerative processes caused by a disease or condition as described in Section (3) hereinafter, but excluding autoimmune diseases. Non-limiting examples include glaucoma, stroke, ischemia, gunshot, and cerebral damage caused by dangerous sports. Thus Cop 1-specific activated T cells, are expected to be useful for the purpose of the present invention and would not have been suggested by known immunotherapy techniques.

The present invention also provides pharmaceutical compositions comprising a therapeutically effective amount of Cop 1 or a Cop 1-related peptide or polypeptide and methods of use of such compositions to promote nerve regeneration or to prevent or inhibit neuronal degeneration in the CNS or PNS, in

which the amount is effective to activate T cells *in vivo* or *in vitro*, wherein the activated T cells inhibit or ameliorate the effects of an injury or disease of the NS.

In the practice of the invention, therapy for amelioration of effects of injury or disease comprising administration of Cop 1-specific activated T cells may optionally be in combination with Cop 1 or a Cop 1-related peptide or polypeptide

Additionally, oral administration of Cop 1 or a Cop 1-related peptide or polypeptide antigen can be combined with active immunization to build up a critical T cell response immediately after injury.

In another embodiment, cell banks can be established to store Cop 1 sensitized T cells for neuroprotective treatment of individuals at a later time, as needed. In this case, autologous T cells may be obtained from an individual. Alternatively, allogeneic or semi-allogeneic T cells may be stored such that a bank of T cells of each of the most common MHC-class II types are present. In case an individual is to be treated for an injury, preferably autologous stored T cells are used, but, if autologous T cells are not available, then cells should be used which share an MHC type II molecule with the patient, and these would be expected to be operable in that individual. The cells are preferably stored in an activated state after exposure to Cop 1 or a Cop 1-related peptide or polypeptide. However, the cells may also be stored in a resting state and activated once they are thawed and prepared for use. The cell lines of the bank are preferably

cryopreserved. The cell lines are prepared in any way which is well known in the art. Once the cells are thawed, they are preferably cultured prior to injection in order to eliminate non-viable cells. During this culturing, the cells can be activated or reactivated using the Cop 1 antigen or peptide as used in the original activation. Alternatively, activation may be achieved by culturing in the presence of a mitogen, such as phytohemagglutinin (PHA) or concanavalin A (preferably the former). This will place the cells into an even higher state of activation. The few days that it takes to culture the cells should not be detrimental to the patient as the treatment in accordance with the present invention may occur any time up to a week or more after the injury in order to still be effective. Alternatively, if time is of the essence, the stored cells may be administered immediately after thawing.

BRIEF DESCRIPTION OF THE FIGURE

Figure 1 is a graph showing neuroprotection of retinal ganglion cells (RGC) following crush injury of optic nerve of adult rats and treatment by active immunization of Cop 1 as compared to immunization with BPS as a control. The mean number of RGC in the retina of Cop 1 treated rats can be seen to be significantly higher than in the retina of control rats.

DETAILED DESCRIPTION OF THE INVENTION

Merely for ease of explanation, the detailed description of the present invention is divided into the

following subsections: (1) Cop 1 Specific Activated T Cells; (2) Cop 1 and Cop 1-Related Peptides and Polypeptides; (3) Therapeutic Uses; (4) Formulations and Administration; (5) Establishment of autologous Cell Banks for T Lymphocytes; (6) Examples; and (7) Discussion.

(1) COP 1 SPECIFIC ACTIVATED T CELLS

Cop 1-specific activated T cells (ATCs) ATCs activated in the presence of Cop 1 or a Cop 1-related peptide or polypeptide, as defined in Section (2). Such ATCs can be used for ameliorating or inhibiting the effects of injury or disease of the CNS or PNS that result in NS degeneration or for promoting regeneration in the NS, in particular the CNS.

The Cop 1-specific activated T cells are preferably autologous, most preferably of the CD4 and/or CD8 phenotypes, but they may also be allogeneic T cells from related donors, e.g., siblings, parents, children, or HLA-matched or partially matched, semi-allogeneic or fully allogeneic donors.

In addition to the use of autologous T cells isolated from the subject, the present invention also comprehends the use of semi-allogeneic T cells for neuroprotection. These T cells may be prepared as short- or long-term lines and stored by conventional cryopreservation methods for thawing and administration, either immediately or after culturing for 1-3 days, to a subject suffering from injury to the central nervous system and in need of T cell neuroprotection.

The use of semi-allogeneic T cells is based on the fact that T cells can recognize a specific antigen epitope

presented by foreign antigen presenting cells (APC), provided that the APC express the MHC molecule, class I or class II, to which the specific responding T cell population is restricted, along with the antigen epitope recognized by the T cells. Thus, a semi-allogeneic population of T cells that can recognize at least one allelic product of the subject's MHC molecules, preferably an HLA-DR or an HLA-DQ or other HLA molecule, and that is specific for a Cop 1 epitope, will be able to recognize the antigens cross-reactive with Cop 1 in the subject's area of NS damage and produce the needed neuroprotective effect. There is little or no polymorphism in the adhesion molecules, leukocyte migration molecules, and accessory molecules needed for the T cells to migrate to the area of damage, accumulate there, and undergo activation. Thus, the semi-allogeneic T cells will be able to migrate and accumulate at the CNS site in need of neuroprotection and will be activated to produce the desired effect.

It is known that semi-allogeneic T cells will be rejected by the subject's immune system, but that rejection requires about two weeks to develop. Hence, the semi-allogeneic T cells will have the two-week window of opportunity needed to exert neuroprotection. After two weeks, the semi-allogeneic T cells will be rejected from the body of the subject, but that rejection is advantageous to the subject because it will rid the subject of the foreign T cells and prevent any untoward consequences of the activated T cells. The semi-allogeneic T cells thus provide an important safety factor and are a preferred embodiment.

It is known that a relatively small number of HLA class II molecules are shared by most individuals in a population. For example, about 50% of the Jewish population express the HLA-DR5 gene. Thus, a bank of specific T cells reactive to Cop 1 epitopes that are restricted to HLA-DR5 would be useful in 50% of that population. The entire population can be covered essentially by a small number of additional T cell lines restricted to a few other prevalent HLA molecules, such as DR1, DR4, DR2, etc. Thus, a functional bank of uniform T cell lines can be prepared and stored for immediate use in almost any individual in a given population. Such a bank of T cells would overcome any technical problems in obtaining a sufficient number of specific T cells from the subject in need of neuroprotection during the open window of treatment opportunity. The semi-allogeneic T cells will be safely rejected after accomplishing their role of neuroprotection. This aspect of the invention does not contradict, and is in addition to the use of autologous T cells as described herein.

The Cop 1-specific activated T cells are preferably non-attenuated, although attenuated Cop 1-specific activated T cells may be used. T cells may be attenuated using methods well known in the art, including but not limited to, by gamma-irradiation, e.g., 1.5-10.0 Rads (Ben-Nun et al, 1981b; Ben-Nun et al, 1982); and/or by pressure treatment, for example as described in U.S. Patent No. 4,996,194 (Cohen et al). In a preferred embodiment the Cop 1-specific activated T cells are isolated as described below. T cells can be isolated and

purified according to methods known in the art (Mor et al, 1995). For an illustrative example, see Section (6), Example 1.

Circulating T cells of a subject which recognize Cop 1 are isolated and expanded using known procedures. In order to obtain Cop 1-specific activated T cells, T cells are isolated and the Cop 1-specific ATCs are then expanded by a known procedure (Burns et al, 1983; Pette et al, 1990; Martin et al, 1990; Schluesener et al, 1985; Suruhan-Dires Keneli et al, 1993, which are incorporated herein by reference in their entirety).

During *ex vivo* activation of the T cells, the T cells may be activated by culturing them in medium to which at least one suitable growth promoting factor has been added. Growth promoting factors suitable for this purpose include, without limitation, cytokines, for instance tumor necrosis factor α (TNF-α), interleukin 2 (IL-2), and interleukin 4 (IL-4).

In one embodiment, the activated T cells endogenously produce a substance that ameliorates the effects of injury or disease in the NS.

In another embodiment, the activated T cells endogenously produce a substance that stimulates other cells, including, but not limited to, transforming growth factor-β (TGF-β), nerve growth factor (NGF), neurotrophic factor 3 (NT-3), neurotrophic factor 4/5 (NT-4/5), brain derived neurotrophic factor (BDNF); interferon-γ (IFN-γ), and

interleukin-6 (IL-6), wherein the other cells, directly or indirectly, ameliorate the effects of injury or disease.

Following their proliferation *in vitro*, the T cells are administered to a mammalian subject. In a preferred embodiment, the T cells are administered to a human subject. T cell expansion is preferably performed using Cop 1 or a Cop 1-related peptide or polypeptide.

Cop 1-activated T cells can be used immediately or may be preserved for later use, e.g., by cryopreservation as described below. Cop 1-specific activated T cells may also be obtained using previously cryopreserved T cells, i.e., after thawing the cells, the T cells may be incubated with Cop 1 or a Cop 1-related peptide or polypeptide, optimally together with peripheral blood lymphocytes (PBL), to obtain a preparation of Cop 1-specific ATCs.

As will be evident to those skilled in the art, the T cells can be preserved, e.g., by cryopreservation, either before or after culture.

Cryopreservation agents which can be used include but are not limited to dimethyl sulfoxide (DMSO) (Lovelock et al, 1959; Ashwood-Smith, 1961), glycerol, polyvinylpyrrolidone (Rinfret, 1960), polyethylene glycol (Sloviter et al, 1962), albumin, dextran, sucrose, ethylene glycol, i-erythritol, D-ribitol, D-mannitol (Rowe et al, 1962), D-sorbitol, i-inositol, D-lactose, choline chloride (Rowe et al, 1962), amino acids (Phan The Tran et al, 1960a), methanol, acetamide, glycerol monoacetate (Lovelock, 1954), inorganic salts (Phan The Tran et al, 1960b; Phan The Tran et al), and DMSO combined

with hydroxyethyl starch and human serum albumin (Zaroulis et al, 1980).

A controlled cooling rate is critical. Different cryoprotective agents (Rapatz et al, 1968) and different cell types have different optimal cooling rates. See, e.g., Rowe et al (1962b); Rowe (1966); Lewis et al, (1967); and Mazur, (1970) for effects of cooling velocity on survival of cells and on their transplantation potential. The heat of fusion phase where water turns to ice should be minimal. The cooling procedure can be carried out by use of, e.g., a programmable freezing device or a methanol bath procedure.

Programmable freezing apparatuses allow determination of optimal cooling rates and facilitate standard reproducible cooling. Programmable controlled-rate freezers such as Cryomed or Planar permit tuning of the freezing regimen to the desired cooling rate curve.

After thorough freezing, cells can be rapidly transferred to a long-term cryogenic storage vessel. In one embodiment, samples can be cryogenically stored in mechanical freezers, such as freezers that maintain a temperature of about -80°C or about -20°C. In a preferred embodiment, samples can be cryogenically stored in liquid nitrogen (-196°C) or its vapor. Such storage is greatly facilitated by the availability of highly efficient liquid nitrogen refrigerators, which resemble large Thermos containers with an extremely low vacuum and internal super insulation, such that heat leakage and nitrogen losses are kept to an absolute minimum.

Considerations and procedures for the manipulation, cryopreservation, and long term storage of T cells can be found, for example, in the following references, incorporated by reference herein: Gorin (1986) and International Atomic Energy Agency (1969).

Other methods of cryopreservation of viable cells, or modifications thereof, are available and envisioned for use, e.g., cold metal-mirror techniques. See Livesey et al (1987); Linner et al (1986); see also U.S. Patent No. 4,199,022 by Senken et al, U.S. Patent No. 3,753,357 by Schwartz, and U.S. Patent No. 4,559,298 by Fahy.

Frozen cells are preferably thawed quickly (e.g., in a water bath maintained at 37-47°C) and chilled immediately upon thawing. It may be desirable to treat the cells in order to prevent cellular clumping upon thawing. To prevent clumping, various procedures can be used, including but not limited to the addition before or after freezing of DNAse (Spitzer et al, 1980), low molecular weight dextran and citrate, citrate, hydroxyethyl starch (Stiff et al, 1983), or acid citrate dextrose (Zaroulis et al, 1980), etc.

The cryoprotective agent, if toxic in humans, should be removed prior to therapeutic use of the thawed T cells. One way in which to remove the cryoprotective agent is by dilution to an insignificant concentration.

Once frozen T cells have been thawed and recovered, they are used to promote neuronal regeneration as described herein with respect to non-frozen T cells. Once thawed, the T cells may be used immediately, assuming that they were

activated prior to freezing. Preferably, however, the thawed cells are cultured before injection to the patient in order to eliminate non-viable cells. Furthermore, in the course of this culturing over a period of about one to three days, an appropriate activating agent can be added so as to activate the cells, if the frozen cells were resting T cells, or to help the cells achieve a higher rate of activation if they were activated prior to freezing. Usually, time is available to allow such a culturing step prior to administration as the T cells may be administered as long as a week after injury, and possibly longer, and still maintain their neuroregenerative and neuroprotective effect.

(2) COP 1 AND COP 1-RELATED PEPTIDES AND POLYPEPTIDES

Pharmaceutical compositions comprising Cop 1 or a Cop 1-related peptide or polypeptide antigen or derivative thereof can be used for preventing or inhibiting the effects of injury or disease that result in NS degeneration or for promoting nerve regeneration in the NS, particularly in the CNS. Additionally, Cop 1 or a Cop 1-related peptide or polypeptide antigen or derivative thereof may be used for *in vivo* or *in vitro* activation of T cells. In one embodiment, methods of promoting nerve regeneration or of preventing or inhibiting the effects of CNS or PNS injury or disease comprise administering Cop 1 or a Cop 1-related peptide or polypeptide antigen or derivative thereof to a mammal wherein the Cop 1 or Cop 1-related peptide or polypeptide antigen or derivative thereof activates T cells *in vivo* to produce a

population of T cells that accumulates at a site of injury or disease of the CNS or PNS.

The composition for use in the present invention can be Cop 1 or a Cop 1-related peptide or polypeptide. For the purpose of the present invention, "Cop 1 or a Cop 1-related peptide or polypeptide" is intended to include any peptide or polypeptide, including a random copolymer, that cross-reacts functionally with myelin basic protein (MBP) and is able to compete with MBP on the MHC class II in the antigen presentation.

The composition may comprise random copolymers comprising a suitable quantity of an amino acid of positive electrical charge, such as lysine or arginine, in combination with an amino acid with a negative electrical charge (preferably in a lesser quantity), such as glutamic acid or aspartic acid, optionally in combination with an electrically neutral amino acid such as alanine or glycine, serving as a filler, and optionally with an amino acid adapted to confer on the copolymer immunogenic properties, such as an aromatic amino acid like tyrosine or tryptophan.

More specifically, the composition for use in the present invention comprises at least one copolymer selected from the group consisting of random copolymers comprising one amino acid selected from each of at least three of the following groups:

(a) lysine and arginine;

(b) glutamic acid and aspartic acid;

(c) alanine and glycine;

(d) tyrosine and tryptophan.

The copolymers for use in the present invention can be composed of L- or D-amino acids or mixtures thereof. As is known by those of skill in the art, L-amino acids occur in most natural proteins. However, D-amino acids are commercially available and can be substituted for some or all of the amino acids used to make the terpolymers and other copolymers of the present invention. The present invention contemplates copolymers containing both D- and L-amino acids, as well as copolymers consisting essentially of either L- or D-amino acids.

In one embodiment of the invention, the copolymer contains four different amino acids, each from a different one of the groups (a) to (d). A preferred copolymer according to this embodiment of the present invention comprises in combination alanine, glutamic acid, lysine, and tyrosine, of net overall positive electrical charge and of a molecular weight of about 2,000 to about 40,000 daltons, preferably of about 2,000 to about 13,000 daltons. The most preferred example is Copolymer 1 (Cop 1) of average molecular weight about 4,700 to about 13,000 daltons. Preferred molecular weight ranges and processes for making a preferred form of Copolymer 1 are described in U.S. Patent No. 5,800,808, the entire contents of which being hereby incorporated in the entirety. It is clear that this is given by way of example only, and that the composition can be varied both with respect to the constituents and relative proportions of the constituents if the above general criteria are adhered to.

Thus, the copolymer may be a polypeptide from about 15 to about 100, preferably from about 40 to about 80, amino acids in length.

In another embodiment, the copolymer contains three different amino acids each from a different one of three groups of the groups (a) to (d). These copolymers are herein referred to as terpolymers.

In one embodiment, the terpolymers for use in the present invention contain tyrosine, alanine, and lysine, hereinafter designated YAK. The average molar fraction of the amino acids in these terpolymers can vary. For example, tyrosine can be present in a mole fraction of about 0.005 to about 0.250; alanine can be present in a mole fraction of about 0.3 to about 0.6; and lysine can be present in a mole fraction of about 0.1 to about 0.5. The average molecular weight is between 2,000 to about 40,000 daltons, and preferably between about 3,000 to about 35,000 daltons. In a more preferred embodiment, the average molecular weight is about 5,000 to about 25,000 daltons. It is possible to substitute arginine for lysine, glycine for alanine, and/or tryptophan for tyrosine.

In another embodiment, the terpolymers for use in the present invention contain tyrosine, glutamic acid, and lysine, hereinafter designated YEK. The average molar fraction of the amino acids in these terpolymers can vary: glutamic acid can be present in a mole fraction of about 0.005 to about 0.300, tyrosine can be present in a mole fraction of about 0.005 to about 0.250, and lysine can be present in a

mole fraction of about 0.3 to about 0.7. The average molecular weight is between 2,000 and about 40,000 daltons, and preferably between about 3,000 and about 35,000 daltons. In a more preferred embodiment, the average molecular weight is about 5,000 to about 25,000 daltons. It is possible to substitute aspartic acid for glutamic acid, arginine for lysine, and/or tryptophan for tyrosine.

In another embodiment the terpolymers for use in the present invention contain lysine, glutamic acid, and alanine, hereinafter designated KEA. The average molar fraction of the amino acids in these polypeptides can also vary. For example, glutamic acid can be present in a mole fraction of about 0.005 to about 0.300, alanine can be present in a mole fraction of about 0.005 to about 0.600, lysine can be present in a mole fraction of about 0.2 to about 0.7. The average molecular weight is between 2,000 and 40,000 daltons, and preferably between about 3,000 and 35,000 daltons. In a more preferred embodiment, the average molecular weight is about 5,000 to about 25,000 daltons. It is possible to substitute aspartic acid for glutamic acid, glycine for alanine, and/or arginine for lysine.

In another embodiment, the terpolymers for use in the present invention contain tyrosine, glutamic acid, and alanine, hereinafter designated YEA. The average molar fraction of the amino acids in these polypeptides can vary. For example, tyrosine can be present in a mole fraction of about 0.005 to about 0.250, glutamic acid can be present in a mole fraction of about 0.005 to about 0.300, and alanine can

be present in a mole fraction of about 0.005 to about 0.800. The average molecular weight is between 2,000 and about 40,000 daltons, and preferably between about 3,000 and about 35,000 daltons. In a more preferred embodiment, the average molecular weight is about 5,000 to about 25,000 daltons. It is possible to substitute tryptophan for tyrosine, aspartic acid for glutamic acid, and/or glycine for alanine.

In a more preferred embodiment, the mole fraction of amino acids of the terpolymers is about what is preferred for Copolymer 1. The mole fraction of amino acids in Copolymer 1 is glutamic acid about 0.14, alanine about 0.43, tyrosine about 0.10, and lysine about 0.34. The most preferred average molecular weight for Copolymer 1 is between about 5,000 and about 9,000 daltons. The activity of Copolymer 1 for the utilities disclosed herein is expected to remain if one or more of the following substitutions is made: aspartic acid for glutamic acid, glycine for alanine, arginine for lysine, and tryptophan for tyrosine.

The molar ratios of the monomers of the more preferred terpolymer of glutamic acid, alanine, and tyrosine, or YEA, is about 0.21 to about 0.65 to about 0.14.

The molar ratios of the monomers of the more preferred terpolymer of glutamic acid, alanine and lysine, or KEA, is about 0.15 to about 0.48 to about 0.36.

The molar ratios of the monomers of the more preferred terpolymer of glutamic acid, tyrosine, and lysine, or YEK, is about 0.26 to about 0.16 to about 0.58.

The molar ratios of the monomers of the more preferred terpolymer of tyrosine, alanine and lysine, or YAK, is about 0.10 to about 0.54 to about 0.35.

The terpolymers can be made by any procedure available to one of skill in the art. For example, the terpolymers can be made under condensation conditions using the desired molar ratio of amino acids in solution, or by solid phase synthetic procedures. Condensation conditions include the proper temperature, pH, and solvent conditions for condensing the carboxyl group of one amino acid with the amino group of another amino acid to form a peptide bond. Condensing agents, for example dicyclohexyl-carbodiimide, can be used to facilitate the formation of the peptide bond. Blocking groups can be used to protect functional groups, such as the side chain moieties and some of the amino or carboxyl groups against undesired side reactions.

For example, the process disclosed in U.S. Patent 3,849,650, can be used wherein the N-carboxyanhydrides of tyrosine, alanine, γ-benzyl glutamate and N ε-trifluoroacetyl-lysine are polymerized at ambient temperatures in anhydrous dioxane with diethylamine as an initiator. The γ-carboxyl group of the glutamic acid can be deblocked by hydrogen bromide in glacial acetic acid. The trifluoroacetyl groups are removed from lysine by 1 molar piperidine. One of skill in the art readily understands that the process can be adjusted to make peptides and polypeptides containing the desired amino acids, that is, three of the four amino acids in Copolymer 1, by selectively eliminating the reactions that

relate to any one of glutamic acid, alanine, tyrosine, or lysine. For purposes of this application, the terms "ambient temperature" and "room temperature" mean a temperature ranging from about 20 to about 26°C.

The molecular weight of the terpolymers can be adjusted during polypeptide synthesis or after the terpolymers have been made. To adjust the molecular weight during polypeptide synthesis, the synthetic conditions or the amounts of amino acids are adjusted so that synthesis stops when the polypeptide reaches the approximate length which is desired. After synthesis, polypeptides with the desired molecular weight can be obtained by any available size selection procedure, such as chromatography of the polypeptides on a molecular weight sizing column or gel, and collection of the molecular weight ranges desired. The present polypeptides can also be partially hydrolyzed to remove high molecular weight species, for example, by acid or enzymatic hydrolysis, and then purified to remove the acid or enzymes.

In one embodiment, the terpolymers with a desired molecular weight may be prepared by a process which includes reacting a protected polypeptide with hydrobromic acid to form a trifluoroacetyl-polypeptide having the desired molecular weight profile. The reaction is performed for a time and at a temperature which is predetermined by one or more test reactions. During the test reaction, the time and temperature are varied and the molecular weight range of a given batch of test polypeptides is determined. The test conditions which provide the optimal molecular weight range for that batch of

polypeptides are used for the batch. Thus, a trifluoroacetyl-polypeptide having the desired molecular weight profile can be produced by a process which includes reacting the protected polypeptide with hydrobromic acid for a time and at a temperature predetermined by test reaction. The trifluoroacetyl-polypeptide with the desired molecular weight profile is then further treated with an aqueous piperidine solution to form a low toxicity polypeptide having the desired molecular weight.

In a preferred embodiment, a test sample of protected polypeptide from a given batch is reacted with hydrobromic acid for about 10-50 hours at a temperature of about 20-28°C. The best conditions for that batch are determined by running several test reactions. For example, in one embodiment, the protected polypeptide is reacted with hydrobromic acid for about 17 hours at a temperature of about 26°C.

As binding motifs of Cop 1 to MS-associated HLA-DR molecules are known (Fridkis-Hareli et al, 1999b), polypeptides of fixed sequence can readily be prepared and tested for binding to the peptide binding groove of the HLA-DR molecules as described in the Fridkis-Hareli et al (1999b) publication. Such peptides would be expected to have similar activity as Cop 1. However, this can readily be determined by testing for their ability to activate T cells in accordance with the present invention. All of this can be done without undue experimentation. Such peptides are also considered to be within the definition of Cop 1-related peptides or

polypeptides and their use is considered to be part of the present invention.

The preferred copolymer for use in the present invention is Copolymer 1, herein referred to also as Cop 1. Copolymer 1 has been approved in several countries for the treatment of multiple sclerosis (MS) under the trade name, COPAXONE®, Glatiramer acetate. COPAXONE® is a trademark of Teva Pharmaceuticals Ltd., Petah Tikva, Israel. Several clinical trials demonstrated that Copolymer 1 is well tolerated with only minor side reactions which were mostly mild reactions at the injection site (Johnson et al, 1995).

(3) THERAPEUTIC USES

The compositions described in Sections (1) through (2) may be used to promote nerve regeneration or to prevent or inhibit secondary degeneration which may otherwise follow primary NS injury, e.g., blunt trauma, such as those caused by participation in dangerous sports, penetrating trauma, such as gunshot wounds, hemorrhagic stroke, ischemic stroke, glaucoma, ischemia, or damages caused by surgery such as tumor excision. In addition, such compositions may be used to ameliorate the effects of disease that result in a degenerative process, e.g., degeneration occurring in either gray or white matter (or both) as a result of various diseases or disorders, including, without limitation: diabetic neuropathy, senile dementias, Alzheimer's disease, Parkinson's Disease, facial nerve (Bell's) palsy, glaucoma, Huntington's chorea, amyotrophic lateral sclerosis (ALS), non-arteritic optic neuropathy, intervertebral disc herniation, vitamin

deficiency, prion diseases such as Creutzfeldt-Jakob disease, carpal tunnel syndrome, peripheral neuropathies associated with various diseases, including but not limited to, uremia, porphyria, hypoglycemia, Sjorgren Larsson syndrome, acute sensory neuropathy, chronic ataxic neuropathy, biliary cirrhosis, primary amyloidosis, obstructive lung diseases, acromegaly, malabsorption syndromes, polycythemia vera, IgA and IgG gammapathies, complications of various drugs (e.g., metronidazole) and toxins (e.g., alcohol or organophosphates), Charcot-Marie-Tooth disease, ataxia telangectasia, Friedreich's ataxia, amyloid polyneuropathies, adrenomyeloneuropathy, Giant axonal neuropathy, Refsum's disease, Fabry's disease, lipoproteinemia, etc.

In a preferred embodiment, the activated T cells or immunization composition comprising Cop 1 or a Cop 1-related peptide or polypeptide of the present invention are used to treat diseases or disorders where promotion of nerve regeneration or prevention or inhibition of secondary neural degeneration is indicated, which are not autoimmune diseases or neoplasias. In a preferred embodiment, the compositions of the present invention are administered to a human subject.

While Cop 1-activated T cells may have been used in the prior art in the course of treatment to develop tolerance to autoimmune antigens in the treatment of multiple sclerosis, the present invention can also be used to ameliorate the degenerative process caused by autoimmune diseases as long as it is used in a manner not suggested by such prior art methods.

Cop 1-activated T cells may also be used to ameliorate the degenerative process caused by neoplasms, without using immunotherapy processes. T cells activated with Cop 1 will accumulate at the site of neural degeneration and facilitate inhibition of this degeneration.

(4) FORMULATIONS AND ADMINISTRATION

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the composition and not deleterious to the recipient thereof.

The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. The carriers in the pharmaceutical composition may comprise a binder, such as microcrystalline cellulose, polyvinylpyrrolidone (polyvidone or povidone), gum tragacanth, gelatin, starch, lactose or lactose monochydrate; a disintegrating agent, such as alginic acid, maize starch and the like; a lubricant or surfactant, such as magnesium stearate, or sodium lauryl sulphate; a glidant, such as colloidal silicon dioxide; a sweetening agent, such as sucrose or saccharin; and/or a flavoring agent, such as peppermint, methyl salicylate, or orange flavoring.

Methods of administration include, but are not limited to, parenteral, e.g., intravenous, intraperitoneal, intramuscular, subcutaneous, mucosal (e.g., oral, intranasal, buccal, vaginal, rectal, intraocular), intrathecal, topical

and intradermal routes. Administration can be systemic c
local.

For oral administration, the pharmaceutical preparation may be in liquid form, for example, solution: syrups or suspensions, or may be presented as a drug proc for reconstitution with water or other suitable vehicle ! use. Such liquid preparations may be prepared by conven means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose deriv or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oily esters, or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates sorbic acid). The pharmaceutical compositions may take form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable exci such as binding agents (e.g., pregelatinized maize starc polyvinyl pyrrolidone or hydroxypropyl methylcellulose) fillers (e.g., lactose, microcrystalline cellulose or ci hydrogen phosphate); lubricants (e.g., magnesium steara talc or silica); disintegrants (e.g., potato starch or starch glycolate); or wetting agents (e.g., sodium laur sulphate). The tablets may be coated by methods well-k the art.

Preparations for oral administration may be s formulated to give controlled release of the active com

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

The compositions may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen free water, before use.

The compositions may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

For administration by inhalation, the compositions for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin, for use in an inhaler or insufflator may be

formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

In a preferred embodiment, compositions comprising Cop 1-activated T cells, a Cop 1 or a Cop 1-related peptide or polypeptide are formulated in accordance with routine procedures as pharmaceutical compositions adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water or saline for injection can be provided so that the ingredients may be mixed prior to administration.

Pharmaceutical compositions comprising Cop 1 or Cop 1-related peptide or polypeptide may optionally be administered with an adjuvant in the usual manner for immunization.

When Cop 1 is introduced orally, it may be mixed with other food forms and consumed in solid, semi-solid, suspension, or emulsion form; and it may be mixed with pharmaceutically acceptable carriers, including water, suspending agents, emulsifying agents, flavor enhancers, and

the like. In one embodiment, the oral composition is enterically-coated. Use of enteric coatings is well known in the art. For example, Lehman (1971) teaches enteric coatings such as Eudragit S and Eudragit L. The Handbook of Pharmaceutical Excipients, 2nd Ed., also teaches Eudragit S and Eudragit L applications. One Eudragit which may be used in the present invention is L30D55.

Cop 1 may also be administered nasally in certain of the above-mentioned forms by inhalation or nose drops. Furthermore, oral inhalation may be employed to deliver Cop 1 to the mucosal linings of the trachea and bronchial passages.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention.

In a preferred embodiment, the pharmaceutical compositions of the invention are administered to a mammal, preferably a human, shortly after injury or detection of a degenerative lesion in the NS. The therapeutic methods of the invention may comprise administration of Cop 1-activated T cells or Cop 1 or Cop 1-related peptide or polypeptide, or any combination thereof. When using combination therapy, Cop 1 may be administered before, concurrently or after administration of Cop 1-activated T cells.

In one embodiment, the compositions of the invention are administered in combination with one or more of the following (a) mononuclear phagocytes, preferably cultured monocytes (as described in PCT publication No. WO 97/09985,

which is incorporated herein by reference in its entirety), that have been stimulated to enhance their capacity to promote neuronal regeneration; (b) a neurotrophic factor such as acidic fibroblast growth factor; and (c) an anti-inflammatory therapeutic substance (i.e., an anti-inflammatory steroid, such as dexamethasone or methylprednisolone, or a non-steroidal anti-inflammatory peptide, such as Thr-Lys-Pro (TKP)).

In another embodiment, mononuclear phagocyte cells according to PCT Publication No. WO 97/09985 and U.S. patent application Serial No. 09/041,280, filed March 11, 1998, are injected into the site of injury or lesion within the CNS, either concurrently, prior to, or following parenteral administration of Cop 1-activated T cells, Cop 1 or a Cop 1-related peptide or polypeptide.

In another embodiment, administration of Cop-activated T cells, Cop 1 or a Cop 1-related peptide or polypeptide, may be administered as a single dose or may be repeated, preferably at 2 week intervals and then at successively longer intervals once a month, once a quarter, once every six months, etc. The course of treatment may last several months, several years or occasionally also through the life-time of the individual, depending on the condition or disease which is being treated. In the case of a CNS injury, the treatment may range between several days to months or even years, until the condition has stabilized and there is no or only a limited risk of development of secondary degeneration. In chronic human disease or Parkinson's disease, the

therapeutic treatment in accordance with the invention may be for life.

As will be evident to those skilled in the art, the therapeutic effect depends at times on the condition or disease to be treated, on the individual's age and health condition, on other physical parameters (e.g. gender, weight, etc.) of the individual, as well as on various other factors, e.g., whether the individual is taking other drugs, etc.

The optimal dose of the therapeutic compositions comprising Cop 1-activated T cells of the invention is proportional to the number of nerve fibers affected by NS injury or disease at the site being treated. In a preferred embodiment, the dose ranges from about 5×10^6 to about 10^7 for treating a lesion affecting about 10^5 nerve fibers, such as a complete transection of a rat optic nerve, and ranges from about 10^7 to about 10^8 for treating a lesion affecting about 10^6-10^7 nerve fibers, such as a complete transection of a human optic nerve. As will be evident to those skilled in the art, the dose of T cells can be scaled up or down in proportion to the number of nerve fibers thought to be affected at the lesion or site of injury being treated.

(5) ESTABLISHMENT OF AUTOLOGOUS CELL BANKS FOR T LYMPHOCYTES

To minimize secondary damage after nerve injury, patients can be treated by administering autologous or semi-allogeneic T lymphocytes sensitized to Cop 1 or a Cop 1-related peptide or polypeptide. As the window of opportunity has not yet been precisely defined, therapy should be

administered as soon as possible after the primary injury to maximize the chances of success, preferably within about one week.

To bridge the gap between the time required for activation and the time needed for treatment, a bank can be established with personal vaults of autologous T lymphocytes prepared for future use for neuroprotective therapy against secondary degeneration in case of NS injury. T lymphocytes are isolated from the blood and then sensitized to Cop 1 or a Cop 1-related peptide or polypeptide. The cells are then frozen and suitably stored under the person's name, identity number, and blood group, in a cell bank until needed.

Additionally, autologous stem cells of the CNS can be processed and stored for potential use by an individual patient in the event of traumatic disorders of the NS such as ischemia or mechanical injury, as well as for treated neurodegenerative conditions such as Alzheimer's disease or Parkinson's disease. Alternatively, semi-allogeneic or allogeneic T cells can be stored frozen in banks for use by any individual who shares one MHC type II molecule with the source of the T cells.

The following examples illustrate certain features of the present invention but are not intended to limit the scope of the present invention.

(6) EXAMPLES

EXAMPLE 1: Accumulation of Activated T Cells in Injured Optic Nerve

Materials

The Cop 1 used in the present examples was the COPAXONE® product, which product was obtained commercially.

Animals

Female Lewis rats were supplied by the Animal Breeding Center of the Weizmann Institute of Science (Rehovot, Israel), matched for age (8-12 weeks) and housed four to a cage in a light and temperature-controlled room.

Media

The T cell proliferation medium contained the following: Dulbecco's modified Eagle's medium (DMEM, Biological 15 Industries, Israel) supplemented with 2 mM L-glutamine (L-Glu, Sigma, USA), 5 x 10^{-5} M 2-mercaptoethanol (2-ME, Sigma), penicillin (100 IU/ml; Biological Industries), streptomycin (100 µ/ml; Biological Industries), sodium pyruvate (1 mM; Biological Industries), non-essential amino acids (1 ml/100 ml; Biological Industries) and autologous rat serum 1% (vol/vol) (Mor et al, 1990). Propagation medium contained: DMEM, 2-ME, L-Glu, sodium pyruvate, non-essential amino acids and antibiotics in the same concentration as above with the addition of 10% fetal calf serum (FCS), and 10% T cell growth factor (TCGF) obtained from the supernatant of concanavalin A-stimulated spleen cells (Mor et al, *supra*, 1990).

T Cell Lines

T cell lines were generated from draining lymph node cells obtained from Lewis rats immunized with Cop 1. The Cop 1 was dissolved in PBS (1 mg/ml) and emulsified with an equal volume of incomplete Freund's adjuvant (Difco Laboratories, Detroit, MI) supplemented with 4 mg/ml *Mycobacterium tuberculosis* (Difco 15 Laboratories, Detroit, MI). The emulsion (0.1 ml) was injected into hind foot pads of the rats. Ten days after the antigen was injected, the rats were killed and draining lymph nodes were surgically removed and dissociated. The cells were washed and activated with the antigen (10 µg/ml) in proliferation medium (described above in Section 6.1.2). After incubation for 72 hours at 37°C, 90% relative humidity and 7% CO_2, the cells were transferred to propagation medium (described above in Section 6.1.2). Cells were grown in propagation medium for 4-10 days before being re-exposed to antigen (10 µg/ml) in the presence of irradiated (2000 red) thymus cells (10^7 cells/ml) in proliferation medium. The T cell lines were expanded by repeated re-exposure and propagation.

Crush Injury of Rat Optic Nerve

Crush injury of the optic nerve was performed as previously described (Duvdevani et al, 1990a). Briefly, rats were deeply anesthetized by i.p. injection of Rompum (xylazine, 10 mg/kg; Vitamed, Israel) and Vetaler (ketamine, 50 mg/kg; Fort Dodge Laboratories, Fort Dodge, IO). Using a binocular operating microscope, a lateral canthotomy was performed in the right eye and the conjunctiva was incised

lateral to the cornea. After separation of the retractor bulbi muscles, the optic nerve was exposed intraorbitally by blunt dissection. Using calibrated cross-action forceps, a moderate crush injury was inflicted on the optic nerve, 2 mm from the eye (Duvdevani et al, 1990b). The contralateral nerve was left undisturbed and was used as a control.

EXAMPLE 2: NEUROPROTECTION BY ANTI-COP 1 T CELLS

T Cells to Cop 1 Protect Neurons from Secondary Degeneration

Immediately after optic nerve injury, rats were injected with anti-Cop 1 T cells. The neurotracer dyé 4-Di-10-Asp was applied to optic nerves distal to the site of the injury, immediately after injury (for assessment of secondary degeneration). Five days after the dye application the retinas were excised and flat mounted. Labeled retinal ganglion cells (RGCs) for three to five randomly selected fields in each retina use counted by fluorescent microscopy. RGC survival in each group of injured nerves was expressed as the percentage of the total number of neurons spared after the primary injury (42% of neurons remained undamaged after the primary injury). The neuroprotective effect of the anti-Cop 1 T cells compared with the PBS was significant ($P<0.02$).

Table 1
Survival of RGCs

Anti-Cop 1 T Cells	26.9 ± 14.4
PBS	10.15 ± 12.1

EXAMPLE 3: IMMUNIZATION WITH COP 1 FOR NEUROPROTECTION

Adult Lewis rats were immunized at the day of the injury with Cop 1 (0.1 mg) emulsified in Complete Freunds Adjuvant (CFA), given intradermally to the two footpads. Subsequently, the animals were fed each for five consecutive days with Cop 1 (1 mg per moulatin). Controls (5 rats) were immunized with CFA free of any antigen and were fed daily with phosphate-buffered saline (PBS). Two weeks after the injury, retinal ganglion cells (RGCs were retrogradely labeled by application of DiI distally to the lesion site (as described in WO 99/60021). Five days later the retinas were excised, and the number of ganglion cells were counted. The results, shown in Fig. 1, were expressed by the mean number of RGC (± SEM). The differences between the number of RGCs in the retina of animals that were treated with Cop 1 was significantly ($P < 0.009$) higher than in control retina.

(7) DISCUSSION OF RESULTS

No cure has yet been found for spinal cord lesions, one of the most common yet devastating traumatic injuries in industrial societies. It has been known for more than 40 years that CNS neurons, unlike neurons of the peripheral nervous system, possess only a limited ability to regenerate after injury. During the last two decades, attempts to promote regeneration have yielded approaches that lead to partial recovery. In the last few years it has become apparent that, although most of the traumatic injuries sustained by the human spinal cord are partial, the resulting functional loss is nevertheless far worse than could be

accounted for by the severity of the initial insult; the self-propagating process of secondary degeneration appears to be decisive.

A substantial research effort has recently been directed to arresting injury-induced secondary degeneration. All attempts up to now have been pharmacologically based, and some have resulted in improved recovery from spinal shock. The present study, in contrast, describes a cell therapy that augments what seems to be a natural mechanism of self-maintenance and leads, after a single treatment, to long-lasting recovery. The extent of this recovery appears to exceed that reported using pharmacological methods.

In most tissues, injury-induced damage triggers a cellular immune response that acts to protect the tissue and preserve its homeostasis. This response has been attributed to macrophages and other cells comprising the innate arm of the immune system. Lymphocytes, which are responsible for adaptive immunity, have not been thought to participate in tissue maintenance. Adaptive immunity, according to traditional teaching, is directed against foreign dangers. Our studies now show, however, that the adaptive T cell immune response can be protective even when there is no invasion by foreign pathogens. In the case of tissue maintenance, the specificity of the T cells is to tissue self-antigens.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying current knowledge, readily modify and/or adapt for various applications such

specific embodiments without undue experimentation and without departing from the generic concept, and, therefore, such adaptations and modifications should and are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments. It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation. The means, materials, and steps for carrying out various disclosed functions may take a variety of alternative forms without departing from the invention. Thus the expressions "means to..." and "means for...", or any method step language, as may be found in the specification above and/or in the claims below, followed by a functional statement, are intended to define and cover whatever structural, physical, chemical or electrical element or structure, or whatever method step, which may now or in the future exist which carries out the recited function, whether or not precisely equivalent to the embodiment or embodiments disclosed in the specification above, i.e., other means or steps for carrying out the same functions can be used; and it is intended that such expressions be given their broadest interpretation.

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WHAT IS CLAIMED IS:

1. A method for preventing or inhibiting neuronal degeneration in the central nervous system or peripheral nervous system, which comprises administering to an individual in need thereof an effective amount of:

(a) activated T cells which have been activated by Cop 1 or a Cop-1 related peptide or polypeptide; or

(b) Cop 1 or a Cop-1 related peptide or polypeptide.

2. A method in accordance with claim 1, for promoting nerve regeneration in the central nervous system or peripheral nervous system for ameliorating the effects of injury or disease.

3. A method in accordance with claim 2, in which said injury comprises spinal cord injury, blunt trauma, penetrating trauma, hemorrhagic stroke, or ischemic stroke.

4. A method in accordance with claim 2, in which said disease is Diabetic neuropathy, senile dementia, Alzheimer's disease, Parkinson's Disease, facial nerve (Bell's) palsy, glaucoma, Huntington's chorea, amyotrophic lateral sclerosis, non-arteritic optic neuropathy, or vitamin deficiency.

5. A method in accordance with claim 2, in which said disease is not an autoimmune disease.

6. A method in accordance with claim 1, wherein said administering step comprises administering to an individual in need thereof an effective amount of activated T

cells which have been activated by Cop 1 or a Cop-1 related peptide or polypeptide.

7. A method in accordance with claim 6, wherein said NS-specific activated T cells are autologous T cells, or allogeneic T cells from related donors, or HLA-matched or partially matched, semi-allogeneic or fully allogeneic donors.

8. A method in accordance with claim 7; wherein said T cells are autologous T cells which have been stored or are derived from autologous CNS cells.

9. A method in accordance with claim 7, wherein said T cells are semi-allogeneic T cells.

10. A method in accordance with claim 1, wherein said administering step comprises administering to an individual in need thereof an effective amount of Cop 1 or a Cop-1 related peptide or polypeptide.

11. A method in accordance with claim 10, wherein said Cop 1 or a Cop-1 related peptide or polypeptide is Cop 1.

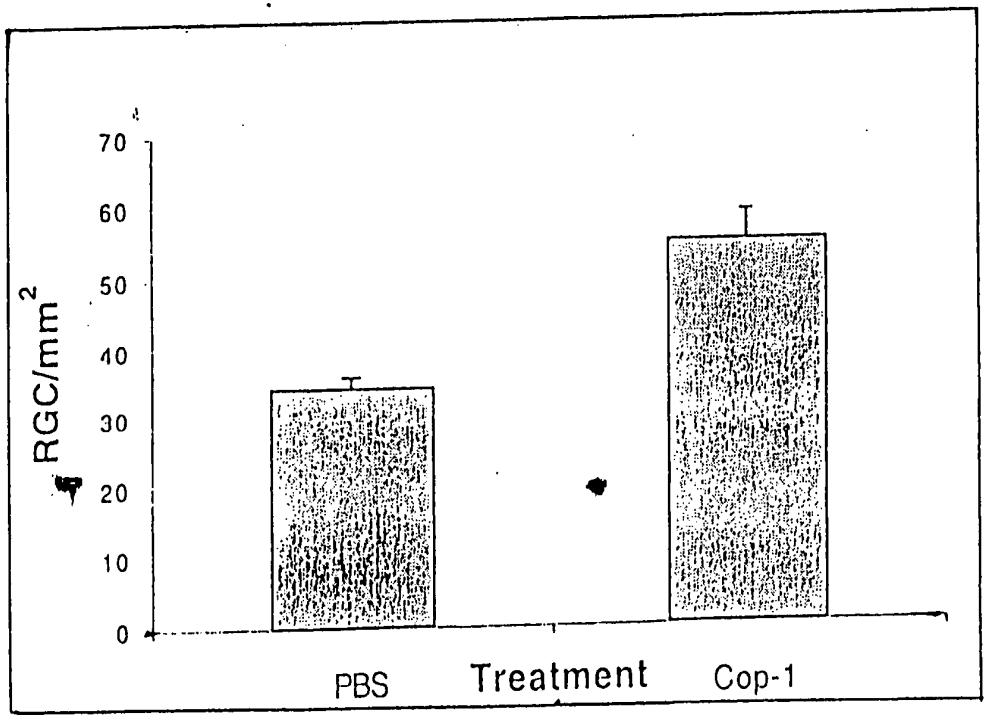
13. A method in accordance with claim 10, in which said Cop 1 or a Cop-1 related peptide or polypeptide is administered intravenously, orally, intranasally, intrathecally, intramuscularlly, intradermally, topically, subcutaneously, mucosally (e.g., orally, intranasally, vaginally, rectally) or bucally.

14. A method for preventing or inhibiting neuronal degeneration in the central nervous system or peripheral nervous system comprising administering to an individual in need thereof an effective amount of Cop 1 or a Cop 1-related

peptide or polypeptide and actively immunizing said individual to build up a critical T cell response.

ABSTRACT OF THE DISCLOSURE

Methods are provided for treating injury to or disease of the central or peripheral nervous system. In one embodiment, treatment is effected using activated T cells that recognize an antigen of Cop 1 or a Cop 1-related peptide or polypeptide to promote nerve regeneration or to prevent or inhibit neuronal degeneration within the nervous system. Treatment involves administering Cop 1 or a Cop 1-related peptide or polypeptide to promote nerve regeneration or to prevent or inhibit neuronal degeneration in the nervous system, either the central nervous system or the peripheral nervous system. The activated T cells, which have been activated by the presence of Cop 1 or a Cop 1-related peptide or polypeptide, can be administered alone or in combination with Cop 1 or a Cop 1-related peptide or polypeptide.



T-TEST
P value = 0.009

FIGURE 1